Peripheral blood stem cells (PBSCs) have been used rarely for allogeneic transplantation because of concerns regarding graft failure and graft-versus-host disease (GVHD). We evaluated the results of allogeneic PBSC transplantation (allo-PBSCPT) in 9 patients with refractory leukemia or lymphoma receiving myeloablative therapy followed by allo-PBSCPT from an HLA-identical sibling donor. Three patients had relapsed 11 to 21 months after allogeneic bone marrow transplantation (allo-BMT) and underwent allo-PBSCCT using the same donor. Six patients received PBSCs as their initial allogeneic transplant. Filgrastim-mobilized PBSCs were collected from the donors in 3 to 4 aphereses and cryopreserved. The apheresis collections contained a median nucleated cell count of 16.5 × 10^6/kg (range, 10.8 to 28.7 × 10^6), 10.7 × 10^6 CD34+ cells/kg (range, 7.5 to 22.5 × 10^6), and 300.0 × 10^6 CD3 cells/kg (range, 127.8 to 1,523.2 × 10^6). The median recovery of CD34+ progenitor cells after freezing, thawing, and washing was 106.4% (range, 36.7% to 132.0%). Patients received filgrastim posttransplant through subcutaneous (SC) every 12 hours administered through continuous infusion for 3 to 4 days. PBSCs were collected using a Cobe Spectra blood cell separator (Cobe BCT, Inc, Lakewood, CO), and the total blood volume processed per apheresis was 4-8 L of each sample were resuspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 1×10^8/kg (range, 10.8 to 28.7 × 10^6), 10.7 × 10^6 CD34+ cells/kg (range, 7.5 to 22.5 × 10^6), and 300.0 × 10^6 CD3 cells/kg (range, 127.8 to 1,523.2 × 10^6). The median recovery of CD34+ progenitor cells after freezing, thawing, and washing was 106.4% (range, 36.7% to 132.0%). All patients received filgrastim posttransplant through engraftment, and cyclosporine and methylprednisolone were used for GVHD prophylaxis. Neutrophil recovery to greater than 0.5 × 10^9/L and greater than 1.0 × 10^9/L occurred at a median of 9 (range, 8 to 10) and 9 days (range 8 to 11) posttransplant, respectively, which was similar to historical controls after allo-BMT and granulocyte-colony-stimulating factor therapy. Platelets recovered to greater than 20 × 10^9/L and greater than 50 × 10^9/L at a median of 12 (range, 8 to 25) and 15 days (range, 11 to 59), respectively, which was significantly more rapid than for the controls (P < .01). Donor cell engraftment was documented by cytogenetics, fluorescence in situ hybridization, and/or restriction fragment length polymorphisms with longest follow-up of 283+ days. Three patients developed grade 2 acute GVHD involving only the skin. Three of four evaluable patients showed limited chronic GVHD. Cryopreserved, filgrastim-stimulated allogeneic PBSCs may be a suitable alternative to allogeneic marrow for transplantation with the advantage of more rapid platelet recovery. Acute GVHD was minimal despite the infusion of 1 log more CD3 cells than with marrow allografts. Further studies are required to assess long-term risks of chronic GVHD.

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DONORS, PATIENTS, AND METHODS

Blood stem cell donors. The blood stem cell donors were HLA-identical siblings. Donor hematopoietic progenitor cells were mobilized into the peripheral blood using filgrastim at a dose of 5 μg/kg subcutaneously (SC) every 12 hours administered through completion of stem cell collection.

Blood stem cell collection, cryopreservation, and thawing. Apheresis was performed daily for 3 days (8 donors) or 4 days (1 donor) beginning on day 4 of filgrastim administration. PBSCs were collected using a Cobe Spectra blood cell separator (Cobe BCT, Inc, Lakewood, CO), and the total blood volume processed per apheresis was between two and three times the donor's blood volume. All apheresis products were frozen at a controlled rate (Model i010; Cryomed, Marietta, OH) with 5% dimethylsulfoxide (DMSO; Cryoserv, Research Industries Corp, Salt Lake City, UT) in 100 mL volumes in two to four freezing bags (Stericen, Inc, Chicago, IL) per apheresis. On day 0, the frozen apheresis products were thawed, pooled, and washed free of DMSO using the Fenwal CS 3000 blood cell separator (Baxter Healthcare Corp, Deerfield, IL) and infused.

Immunophenotyping. The apheresis products, control marrow harvests, and the recipients' peripheral blood samples posttransplant were analyzed by two- or three-color flow cytometry. Cells (1 × 10^6) of each sample were resuspended in 100 μL of phosphate-buffered saline (PBS) containing 1% bovine serum albumin and stained with monoclonal antibodies (Becton Dickinson, Mountain View, CA) for CD34-FITC (clone 8G12), CD38-PE (clone HB7) and HLA-DR-PerCP (clone L243) at 10 μL each. Isotype controls combined with specific antibodies were used for setting amplification.
and compensation of the flow cytometer. The following combinations of control settings were used: (IgG1-FITC, IgG1-PE, IgG2a-PerCP), (CD34-FITC, IgG1-PE, IgG2a-PerCP), (IgG1-FITC, CD38-PE, IgG2a-PerCP), and (IgG1-FITC, IgG1-PE, HLA-DR-PerCP). Cells were analyzed on a FACScan (Becton Dickinson, Palo Alto, CA) equipped with a 15 mW Argon ion laser tuned emitting at 488 nm. FITC-immunofluorescence (FL 1), PE-immunofluorescence (FL 2), and PerCP-immunofluorescence (FL 3) were measured at 530, 585, and 650 nm, respectively. All fluorescence parameters were amplified logarithmically. The scatter parameters (90° side and forward scatter) were amplified linearly. A Hewlett-Packard 300 computer system (Hewlett-Packard, Roseville, CA) was used for processing of list mode data. CD34+ cells were quantified as a proportion of the patients had marrow immunophenotyping; all such data available are reported.

**Table 1. Patient Characteristics, Engraftment, and Chimerism**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Patient Age/Gender</th>
<th>Donor Age/Gender</th>
<th>Preparative Regimen</th>
<th>Days to Neutrophils</th>
<th>Days to Platelets</th>
<th>Latest Chimerism/MRD Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AML</td>
<td>24/F</td>
<td>28/M</td>
<td>CY/TBI</td>
<td>&gt;0.5 x 10^9/L</td>
<td>&gt;0.5 x 10^9/L</td>
<td>20/20 46.6XV 283* Donor</td>
</tr>
<tr>
<td>2</td>
<td>AML</td>
<td>46/M</td>
<td>48/M</td>
<td>TBI</td>
<td>10</td>
<td>11</td>
<td>10</td>
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<td>3</td>
<td>Hodgkin's</td>
<td>24/M</td>
<td>22/F</td>
<td>CBV</td>
<td>9</td>
<td>9</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>Lymphoma</td>
<td>35/F</td>
<td>28/M</td>
<td>TBI</td>
<td>8</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Lymphoma</td>
<td>51/F</td>
<td>46/M</td>
<td>CY/TBI</td>
<td>8</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>Lymphoma</td>
<td>59/F</td>
<td>50/F</td>
<td>TBI</td>
<td>9</td>
<td>9</td>
<td>17</td>
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<td>Lymphoma</td>
<td>35/M</td>
<td>41/F</td>
<td>TBI</td>
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<tr>
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<td>AML</td>
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<td>36/M</td>
<td>40/M</td>
<td>CY/TBI</td>
<td>10</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P &lt; .01</td>
</tr>
</tbody>
</table>

Abbreviations: CY/TBI, cyclophosphamide, total body irradiation; TBI, Thiotepa, busulfan; CY, CBV, CY, BCNU, etoposide; NS, not significant.

* Patients no. 1, 2, and 5 had clonal cytogenetic abnormalities while in relapse. Numbers in brackets refer to transplant day of latest study.

**RESULTS**

**Immunophenotypic characterization of the apheresis collections.** The total apheresis collections had a mean num-
Circulating hematopoietic progenitors were serially assessed by flow cytometry posttransplant. The numbers of CD34+ cells and more primitive subsets of CD34+ cells peaked early after allo-PBSCT, between days 10 and 13, while the patients were receiving filgrastim. The number of circulating hematopoietic progenitors decreased after filgrastim was discontinued but recovered over 50 to 56 days posttransplant (Fig 1).

Chimerism and minimal residual disease. All evaluable patients had complete donor chimerism in marrow samples as determined by cytogenetics and/or RFLP (Table 1). Patient no. 1 also had 85 of 85 interphase cells positive for X and Y chromosomes by FISH at day 60.

In Patient no. 1, leukemia cell metaphases identified by the t(16;17) were not found posttransplant (Table 1). However, in Patient no. 2, despite a marrow in morphologic remission, cytogenetic abnormalities consistent with the original leukemia were detected in a small number of cells postransplant. During first remission, the karyotype was 46.XY. At the time of initial relapse, the karyotype was 45.XY.inv(3),−7, but no −7 was present after reinduction was attempted with conventional chemotherapy pretransplant. At 3 weeks postransplant, 1 of 30 metaphases had the inv(3). At 2, 4, 8, and 19 weeks postransplant, 3 of 500 (0.6%), 5 of 500 (1.0%), 4 of 500 (0.8%), and 4 of 500 (0.8%) interphase cells, respectively, had a single chromosome 7 by FISH. These levels are within background range for this probe (mean, 1.17% ± 0.67% with −7 for normal peripheral blood buffy coat cells). Patient no. 5 had no evidence of the Philadelphia chromosome as late as day 83 postransplant, and the bcr gene was germline by Southern blot analysis. GVHD. Three of the nine patients developed isolated stage 3 acute GVHD of the skin (Table 3); two responded rapidly to treatment with high-dose methylprednisolone and one resolved with topical therapy. Patient no. 1 had no acute GVHD after either the first or second transplant, patient no. 5 had grade 2 cutaneous and visceral GVHD after his first transplant and only skin involvement after the second transplant, and patient no. 9 had skin involvement after the first transplant and no GVHD after the second transplant to date. None of the allo-PBSCT recipients required ATG. For the control group, the rate of grades 2 to 4 acute GVHD was 70%, and 94% of the patients who developed acute GVHD did so within 1 month postransplant.

Five patients have been observed for at least 100 days postransplant and three have developed chronic GVHD (Table 3). Two have localized involvement and are on no treatment or topical therapy alone.

Outcome. One patient died of parainfluenza pneumonia and sepsis 82 days after allo-PBSCT. No evidence of
lymphoma was found at autopsy. The remainder of the patients are alive 24+ to 326+ days posttransplant. One patient with lymphoma achieved a partial remission, and the remaining patients are all in complete remission. For patient no. 1 the remission duration after allo-PBSCT has been 4 months longer than that after allo-BMT.

**DISCUSSION**

The long-term reconstitutive capability of blood-derived hematopoietic stem cells has not yet been proven after allogeneic transplantation in humans. Using sex-mismatched murine transplants and a molecular probe for Y-chromosome-specific DNA sequences, Moloney et al19 showed

**Table 3. GVHD and Outcome**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Maximum Acute GVHD</th>
<th>Day Onset</th>
<th>Chronic GVHD</th>
<th>Day Onset</th>
<th>Survival</th>
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<tbody>
<tr>
<td></td>
<td>Skin</td>
<td>Liver</td>
<td>Gut</td>
<td>Overall</td>
<td>Day Onset</td>
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<tr>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>—</td>
</tr>
<tr>
<td>4</td>
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<td>0</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<td>0</td>
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</table>

Abbreviations: CR, complete remission; PR, partial remission.
that granulocyte colony-stimulating factor (G-CSF)–primed blood stem cell transplants are capable of durably reconstituting the hematopoiesis. Carbouell et al reported that DLA-mismatched canine blood stem cell transplantation resulted in long-term donor type engraftment for up to 3.2 years posttransplant. In this study, we report nine patients with successful lymphohematopoietic engraftment of allogeneic, HLA-identical PBSCs after myeloablative therapy. With a longest follow-up of 326+ days after allo-PBSCT, all three cell lineages of the hematopoietic system continue to function, indicating a sufficient self-renewal capacity of frozen/thawed, blood-derived stem cells. When exposed to stem cell suppressive drugs such as methotrexate or ganciclovir, the hematopoietic function after allo-PBSCT did not seem to be more susceptible than after allo-BMT.

Low levels of the CD34⁺CD38⁻ subset, which includes putative reconstituting stem cells, were present and peaked in the early recovery phase after allo-PBSCT. This is consistent with a recent study demonstrating that G-CSF– and granulocyte-macrophage colony-stimulating factor (GM-CSF)–mobilized apheresis products contain CD34⁺ Thy-1⁺ and CD38⁻ subsets.

Efficient blood stem cell mobilization in normal donors using filgrastim allows apheresis collections to match or exceed a single marrow harvest in progenitor content without exposing the donor to the risks of anesthesia and a marrow harvest. Sheridan et al reported a median 58-fold increase in colony-forming unit granulocyte-macrophage (CFU-GM) over pretreatment values with filgrastim at 12 µg/kg/d. The optimal cell dose and number of CD34⁺ cells for allogeneic PBSCT is unknown. Matsunaga et al suggested that an engrafting dose of PBSCs could be collected in as little as one apheresis from donors receiving filgrastim at a dose of 2.5 µg/kg for 6 successive days followed by 5 µg/kg for the following 4 days. Fritsch et al also reported that, by a single leukapheresis, sufficient PBSCs can be collected from a cytokine-stimulated normal donor to ensure hematopoietic engraftment in an adult recipient. Our data show that, using filgrastim at 6 µg/kg SC twice daily, a target engraftment dose of 4 × 10⁶ CD34⁺ cells/kg could also be collected with 2 or less aphereses. But, even in normal donors, some variability in cytokine-mobilized and apheresis-derived stem cell yield has to be taken into consideration.

Neutrophil recovery was rapid in our patients after allogeneic PBSCT, but it did not occur any earlier than for the historical control group that did not receive methotrexate for GVHD prophylaxis after allo-BMT. In contrast, platelet recovery was more rapid in our patients after allo-PBSCT than for the allo-BMT historical control group, similar to the experience with autologous PBSCT. Filgrastim itself does not affect platelet recovery after allogeneic or autologous BMT. However, filgrastim does increase numbers of circulating megakaryocyte progenitors in PBSC donors, and this has been associated with an accelerated platelet recovery in recipients of autologous filgrastim-stimulated PBSCs.

It is uncertain whether infusion of larger numbers of lymphocytes present in the PBSC allograft will improve immune reconstitution posttransplant. For autologous recipients, Roberts et al reported that total T cells and CD4⁺ cell number increased more rapidly after PBSCT than after BMT, whereas Henon et al found little difference in immunologic recovery between PBSC and marrow recipients. Prolonged use of cyclosporine may obviate any advantage of PBSCT for immune reconstitution, but further studies are warranted to fully investigate this.

Whether there is a linear relationship between the number of T cells infused and the development of GVHD as postulated by Owens and Santos in murine studies or whether above a certain number of T cells a plateau is reached at which MHC disparity is the major GVHD inducing factor remain to be determined. Nonetheless, despite the large numbers of T cells administered with the allo-PBSCT, GVHD was mild or nonexistent in our patients. However, the observed presence of GVHD in target organs such as skin is in agreement with data reported by Sanders et al, who noticed GVHD after second transplant even if not present after the first transplant. In our experience of two evaluable patients who underwent a second allo-PBSCT, acute GVHD was less than after first allo-BMT or nonexistent. Eckardt et al have also noted that cryopreservation of allogeneic marrow reduced the risk of acute GVHD, possibly through selective depletion of or induction of anergy in GVHD-inducing cells. How cryopreservation affects the alloreactivity of PBSCs needs to be investigated further.

The PBSC collections also contained a high number of CD3⁺CD4⁺CD8⁺ (data not shown) and CD3⁻CD56⁻ subsets that may include the natural suppressor cells thought to be responsible for inhibition of GVHD effect early posttransplant, but the numbers or ratios of the lymphocyte subsets for optimal activity has not been determined.

It is noteworthy in our study that, at the time of writing, the duration of post allo-PBSCT remission exceeds the prior post allo-BMT remission in patient no. 1 by 4 months, leading to speculation regarding a possible additional graft-versus-leukemia (GVL) effect after allo-PBSCT. T lymphocytes present in the allogeneic graft confer a GVL effect, as evidenced by the increased relapse rate after T-cell–depleted BMT and the induction of remission by infusion of donor leukocytes in patients relapsing posttransplant. With increased numbers of T lymphocytes and natural killer cells present in PBSCT collections, it remains to be determined whether these cells are able to confer an enhanced GVL effect.

In conclusion, a number of considerations might favor the circulating blood as a stem cell source for allografting: (1) shorter duration of posttransplant aplasia, (2) faster hematopoietic and/or immune-reconstitution, and (3) a potentially more pronounced GVL effect. In addition, general anesthesia is not necessary for collection and the procedure results in less discomfort for the donor. This experience shows that allo-PBSCT is feasible and warrants further clinical trials. With confirmation of the safety and efficacy of allo-PBSCT, this new technology has the potential to become the basis for an unrelated blood stem cell donor program that from a logistical point of view might not be very different from current apheresis donations.
ACKNOWLEDGMENT

We are indebted to the BMT nurses, clinical nurse specialists, and laboratory personnel for their excellent patient care and technical assistance.

NOTE ADDED IN PROOF

As of December 15, 1994, the total number of evaluable patients with refractory leukemia, lymphoma, or MDS who received a PBSC transplant from their HLA-identical sibling donors increased to 16. The longest follow-up with proven donor chimerism (RFLP) is 463+ days, with a median follow-up of 121 days. The median time to ANC greater than 500/µL and ANC greater than 1,000/µL was 9 days (range, 8 to 10 days) and 9 days (range, 8 to 11 days), respectively, and 12 days (range, 8 to 87+ days) and 15 days (range, 8 to 87+ days) to platelets greater than 20,000/µL, and greater than 50,000/µL, respectively. Platelet recovery after allo-PBSCT was significantly faster than after allo-BMT (P < .01). The actuarial rate of grades 2-4 acute GVHD was 47%; 2 of 8 evaluable patients developed limited chronic GVHD and 3 of 8 evaluable patients developed clinically extensive chronic GVHD. Four patients underwent an allo-PBSCT after relapse from allo-BMT. In 3 patients, acute GVHD was less than after allo-BMT; in 1 patient, acute GVHD became more severe. In patient no. 1, the duration of post-allo-PBSCT remission exceeds the prior post-allo-BMT remission by 8 months.

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Allogeneic blood stem cell transplantation for refractory leukemia and lymphoma: potential advantage of blood over marrow allografts [see comments]

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